

Immune Function Changes During a Spaceflight-analog Undersea Mission

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BACKGROUND

There is ample evidence to suggest that space flight leads to immune system dysregulation. This may be a result of microgravity, confinement, physiological stress, radiation, environment or other mission-associated factors. It is attractive to utilize ground-based spaceflight analogs as appropriate to investigate this phenomenon. For spaceflight-associated immune dysregulation (SAID), the authors believe the most appropriate analogs might be NEEMO (short duration, Shuttle analog), Antarctic winter-over (long-duration, ISS analog) and the Houghton Mars Project in the Canadian Arctic (intermediate-duration). Each of these analogs replicate isolation, mission-associated stress, disrupted circadian rhythms, and other aspects of flight thought to contribute to SAID. To validate NEEMO as a flight analog with respect to SAID, a pilot study was conducted during the NEEMO-12 and 13 missions during 2007. Assays were performed that assessed immune status, physiological stress and latent viral reactivation. Blood and saliva samples were collected at pre-, mid-, and post-mission timepoints.

SPACEFLIGHT ANALOGS

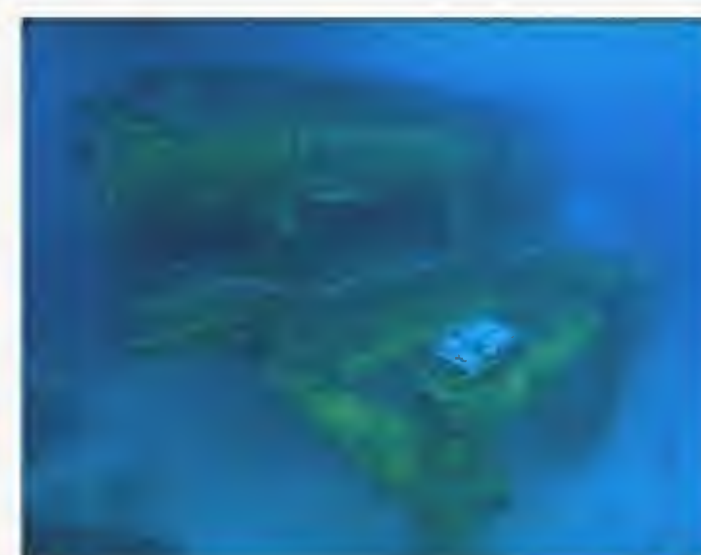
- Closed chamber confinement (psychological)
- Antarctic winter-over (stress, isolation, psychological)
- Houghton-Mars Project (mission stress, isolation, psychological, circadian rhythms)
- Bed rest, HDT, extended duration (bone, muscle)
- BR+hyper-G (bone/muscle + launch/re-entry stress)
- BR+ Artificial Gravity (bone/muscle + countermeasure)
- NEEMO (everything but microgravity/radiation?)

NEEMO-12/13 ASSAYS

- Peripheral leukocyte subset distribution
- T cell function
- Cytokine production profiles
- Latent viral reactivation
- Number and function of viral specific T cells
- Stress hormones (plasma, urine, saliva)
- Viral DNA (urine, saliva)



International Space Station



Aquarius (NEEMO) Undersea Station



NEEMO 12 Blood Collection



NEEMO 12 Crewmembers



NEEMO 13 Crewmembers

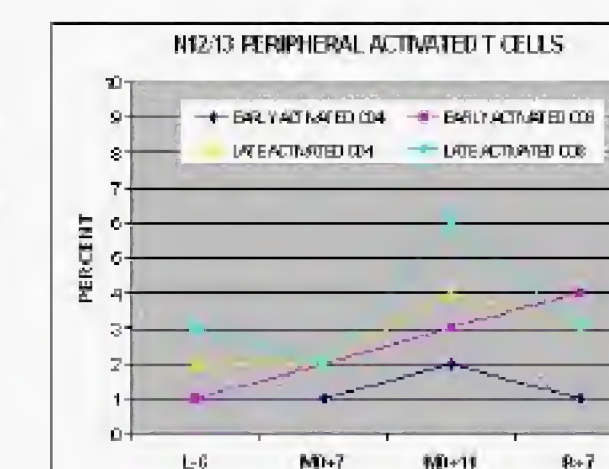
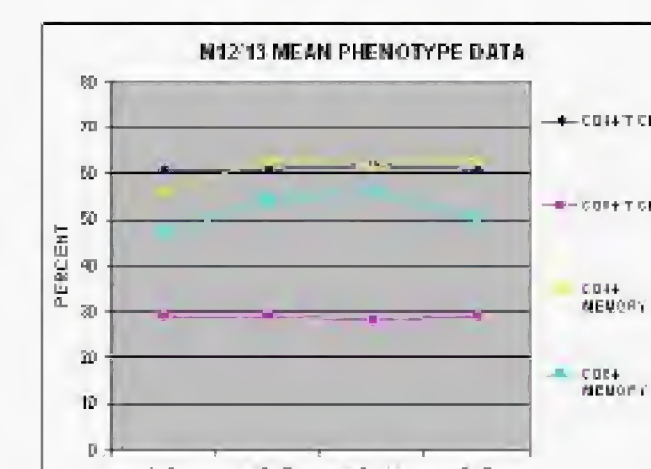
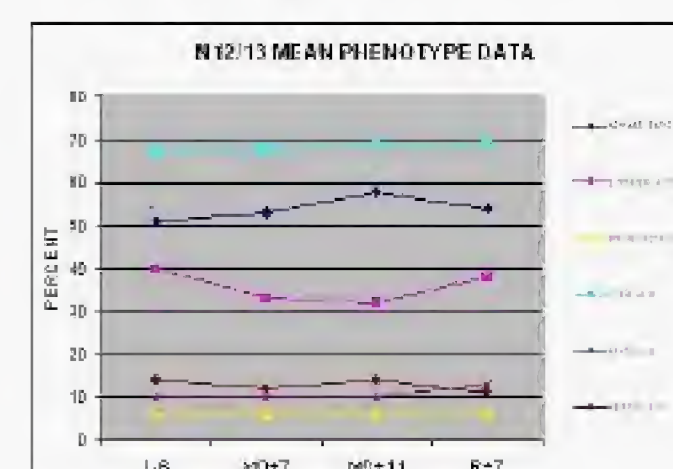


NEEMO 12 Crewmembers outside Aquarius

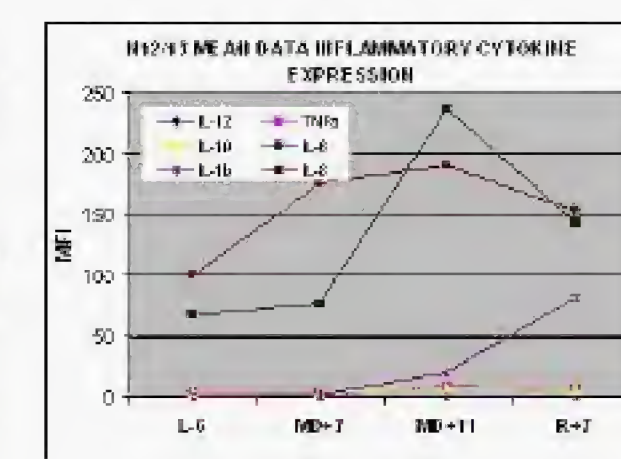
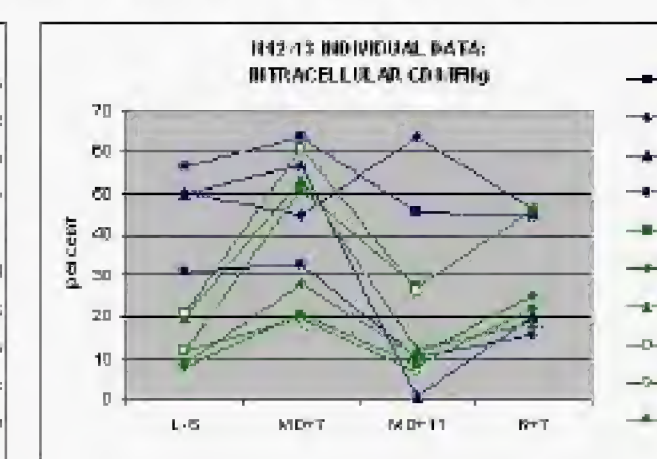
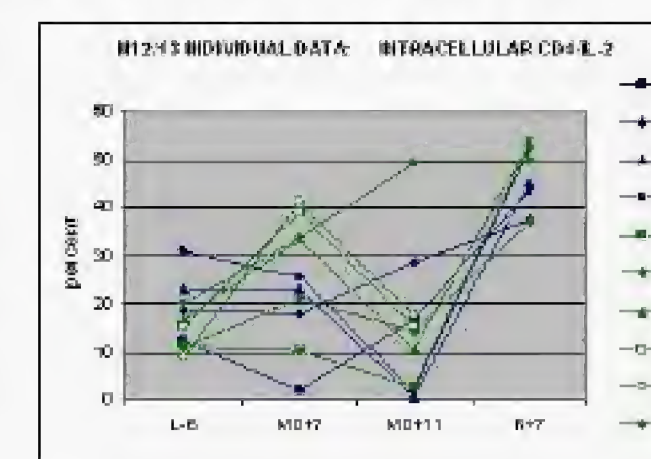
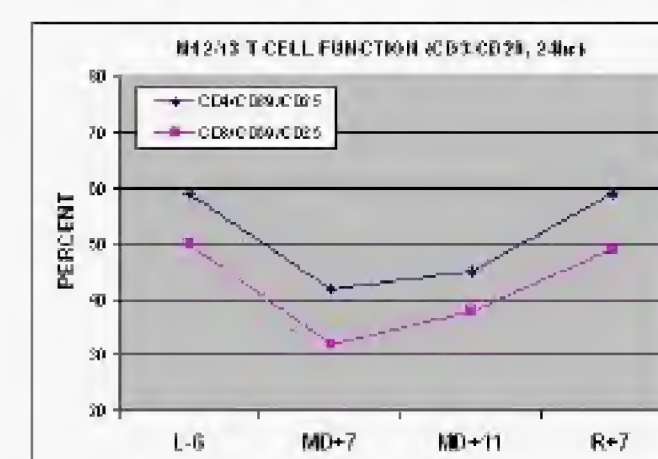
CONCLUSION

The data revealed minimal changes in peripheral leukocyte subsets, as would be expected from healthy subjects in an adverse environment in the absence of actual illness. There were however, dramatic alterations in T cell function. Intracellular cytokine profiles within T cell subsets were altered, and generalized T cell function was diminished during the missions, in a similar fashion to that observed post-flight in ISS crewmembers. Serological evidence of EBV reactivation was observed in 50% of the subjects. As evidence of latent VZV reactivation, salivary VZV DNA was detected in 2 of the 4 NEEMO-12 subjects. Plasma cortisol was elevated in some of the NEEMO subjects. Salivary cortisol increased during the mission compared to pre- and post-mission values. Taken together, the pilot study data seem to validate the NEEMO analog as being appropriate to replicate some aspects of SAID observed during short duration Shuttle flights. In addition, the ease of utility and high-fidelity of the analog make it attractive for rapid investigations. However, to investigate SAID associated with prolonged missions (a key element to determining clinical risk for exploration class missions) another analog would be required.

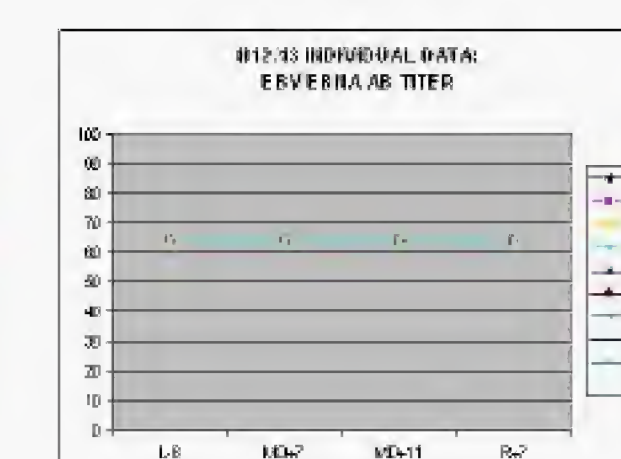
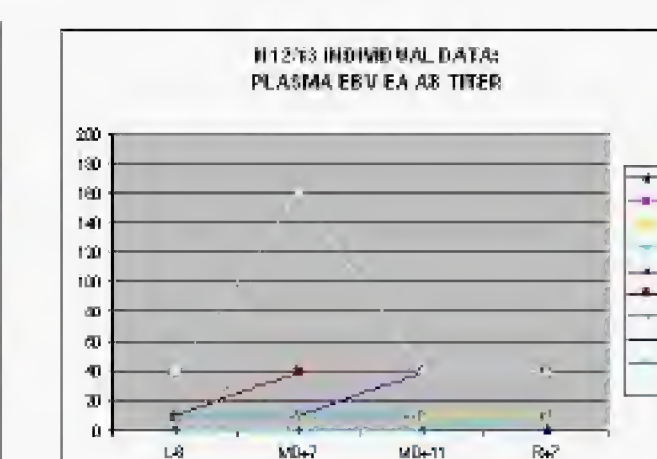
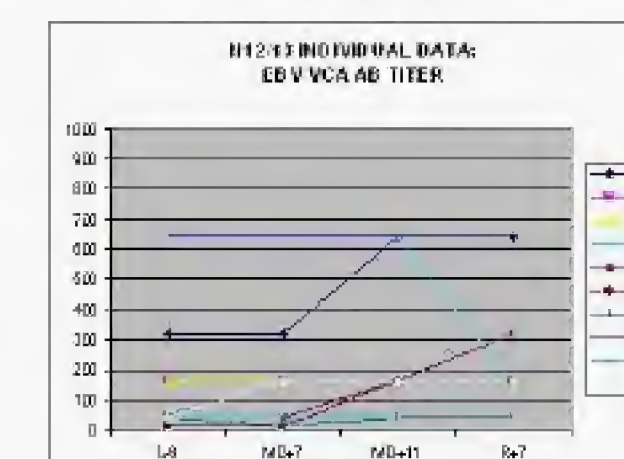
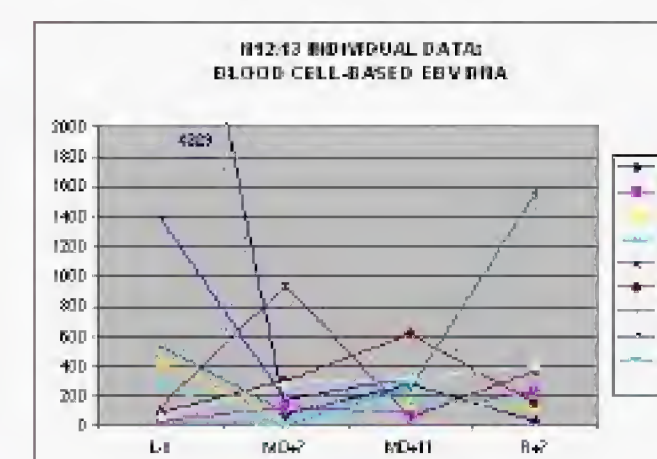
I. Peripheral leukocyte subsets: The primary leukocyte and lymphocyte subsets were found to be unchanged during the NEEMO mission (A, B). Levels of constitutively activated T cells were elevated during the mission, and trended to resolve post-mission (C).



II. T cell function and cytokine profiles. (A) T cell function (CD69+CD25+ expression following CD3/CD28 T cell activation during whole blood culture) was found to be depressed during NEEMO missions, as compared to pre-mission baseline and post-mission recovery data. This is very similar to post-flight data from ISS crewmembers. (B/C) Individual data demonstrating levels of T cells capable of being stimulated to produce cytokine (CD4/IL-2, CD8/IFN γ respectively) are altered during NEEMO missions. Performed via whole blood stimulation with PMA+ionomycin. (D) Secreted monocyte cytokine profiles following whole blood stimulation with LPS, detected via the cytometric bead array method.



III. EBV REACTIVATION. (A) Determination of EBV DNA in peripheral B cells (viral load) by quantitative PCR (N12, N13 individual subject data). (B-D) Peripheral blood levels of antibody titers for (B) EBV-VCA, (C) EBV-EA and (D) EBV-EBNA (N12, N13 individual subject data). Increases in EBV VCA represent lytic viral reactivation, as more virus are presented to the immune system.



IV. VZV REACTIVATION AND STRESS HORMONE LEVELS. (A) Latent VZV reactivation as detected the presence of VZV DNA in saliva, detected by quantitative PCR. Sampling points are different between the two missions, and the additional mid-mission samples for N13 will be analyzed soon via a data sharing agreement. (B, C) Cortisol levels measured in plasma (B, individual crew data); and saliva (C, mean N12/13 data). The data vary among crewmembers, but generally support mid-mission increases in physiologic stress for most crewmembers during the NEEMO missions.

N12/13: SALIVARY VZV DNA (COPIES/mL)															
L-6	MD+7	MD+11	MD+15	MD+19	MD+23	MD+27	MD+31	MD+35	MD+39	MD+43	MD+47	MD+51	MD+55	MD+59	R-7
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

